Heterogeneity of chlorophyll fluorescence over thalli of several foliose macrolichens exposed to adverse environmental factors: Interspecific differences as related to thallus hydration and high irradiance

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Abstract

Spatial heterogeneity of chlorophyll (Chl) fluorescence over thalli of three foliose lichen species was studied using Chl fluorescence imaging (CFI) and slow Chl fluorescence kinetics supplemented with quenching analysis. CFI values indicated species-specific differences in location of the most physiologically active zones within fully hydrated thalli: marginal thallus parts (Hypogymnia physodes), central part and close-to-umbilicus spots (Lasallia pustulata), and irregularly-distributed zones within thallus (Umbilicaria hirsuta). During gradual desiccation of lichen thalli, decrease in Chl fluorescence parameters (F0 - minimum Chl fluorescence at point O, F∞ - maximum Chl fluorescence at P point, Φq - effective quantum yield of photochemical energy conversion in photosystem 2) was observed. Under severe desiccation (>85 % of water saturation deficit), substantial thallus parts lost their apparent physiological activity and the resting parts exhibited only a small Chl fluorescence. Distribution of these active patches was identical with the most active areas found under full hydration. Thus spatial heterogeneity of Chl fluorescence in foliose lichens may reflect location of growth zones (pseudomeristems) within thalli and adjacent newly produced biomass. When exposed to high irradiance, fully-hydrated thalli of L. pustulata and U. hirsuta showed either an increase or no change in F0, a decrease in F∞, Distribution of Chl fluorescence after the high irradiance treatment, however, remained the same as before the treatment. After 60 min of recovery in the dark, F0 and F∞ did not recover to initial values, which may indicate that the lichen used underwent a photoinhibition. The CFI method is an effective tool in assessing spatial heterogeneity of physiological activity over lichen thalli exposed to a variety of environmental factors. It may be also used to select a representative area at a lichen thallus before application of single-spot fluorometric techniques in lichens.

Additional key words: chlorophyll fluorescence imaging; desiccation; high irradiance; high light; Hypogymnia physodes; Lasallia pustulata; lichen; photosynthetic parameters; Umbilicaria hirsuta.

Introduction

The method of chlorophyll (Chl) fluorescence imaging (Lang et al. 1994) has already met an increasing number of applications in the laboratory and in the field (e.g., Lichtenhaler and Miehé 1997, Lichtenthaler et al. 1997). Using Chl fluorescence imaging (CFI), several problems have been studied in higher plants, e.g., heterogeneity of intercellular CO2 concentration (Ci) (Meyer and Genty 1998), patchiness of stomatal closure during ongoing water stress (Meyer and Genty 1999), early detection of water and temperature stress (Lang et al. 1996), photoinhibition of photosynthesis (Osmond et al. 1999), effects of herbicides (Lichtenhaler et al. 1997) and viral infection (Osmond et al. 1998) on decrease of leaf photosynthetic performance. While in higher plants heterogeneity of Chl fluorescence and net photosynthetic rate over a leaf blade has been documented many times (Šesták and Šiffl 1997), information on the heterogeneity in lower plants and lichens is almost lacking. Jensen and

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Symbols and abbreviations: CFI - chlorophyll fluorescence imaging; Chl - chlorophyll; Fv - chlorophyll fluorescence at P point of Chl fluorescence kinetics, Fv'/Fm' - variable chlorophyll fluorescence; α - gross photosynthetic rate, PPDF - photosynthetic photon flux density; PS2 - photosystem 2; WSD - water saturation deficit; Φq - effective quantum yield of photochemical energy conversion in photosystem 2.

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Siebke (1997) used CFI for screening of Chl presence in lichen thalli and simple assessment of physiologically active thallus parts. Another attempt to estimate Chl fluorescence heterogeneity in the field was made by Schroeter et al. (1997) who reported significant differences in quantum yield of photochemical energy conversion of photosystem 2 ($\Phi_2$) between central and marginal parts of Buellia frigida thallus. To our best knowledge, no attempt has yet been made to estimate changes in Chl fluorescence heterogeneity over a lichen thallus as induced by changes in physical factors of environment (e.g., air temperature, water status of thallus). Since lichens are organisms without specialised water conducting tissue and efficient control mechanisms of water loss, one may expect higher heterogeneity in Chl fluorescence distribution over a lichen thallus than in a leaf blade of higher plants. Also the non-uniform distribution of photobiont cells (unicellular algae or cyanobacteria) within lichen thallus and the age-related differences in optical properties of upper cortex may contribute to the Chl fluorescence heterogeneity.

Considering the above facts, we hypothesised that heterogeneity of Chl fluorescence over lichen thalli should be high even when measured under standardised conditions (optimum hydration of thallus, constant air temperature). Another working hypothesis to prove was that there should be inter-specific differences in Chl fluorescence distribution over lichen thalli that reflect different growth patterns. In addition, the changes in Chl fluorescence distribution over thalli ongoing with desiccation and high irradiance stress were recorded and analysed. The aim of our paper was to test the above hypotheses and to enlarge the applicability of CFI to stress physiology in lichens.

**Materials and methods**

**Lichens**: Thalli of three foliose lichen species were collected from field sites in the naturally desiccated state in January 2000. *Hypogymnia physodes* was collected from bark of orchard trees in close vicinity to Moravany village, 5 km south of Brno, Czech Republic. Thalli of *Lasallia pustulata* and *Umbilicaria hirsuta* were collected from perpendicular granitic rock walls in the Oslava river valley near the village Ketkovice, 30 km west of Brno, Czech Republic. All collected thalli were transferred to a laboratory and stored at 5°C. Prior to the measurements, the thalli were covered by wet paper and re-hydrated for 2 h at room temperature.

**Chl fluorescence** was measured on dark-adapted (10 min), fully hydrated lichen thalli, and after gradual desiccation on thalli showing a different water saturation deficit (WSD). The Chl fluorescence signal was measured using a kinetic fluorometric CCD camera *FluorCam* (Photon Systems Instruments, Brno, Czech Republic). Chl fluorescence was induced by imposing a 1 s pulse of weak excitation irradiance ($\lambda = 650$ nm, 120 µmol m$^{-2}$ s$^{-1}$) on the lichen thallus. Fast changes in Chl fluorescence of the whole thallus during the irradiance-induced Chl fluorescence kinetics (Kautsky effect) were recorded with the time resolution of 0.04 s and stored as an image file using a *FluorCam* software. Further analysis of the image file consisted of re-colouring original monochrome image (grey scale) into false colours and identification of maximum and minimum Chl fluorescence signal within a single thallus. For this purpose, the peak Chl fluorescence $F_P$ was chosen. The minimum-to-maximum span of $F_P$ was divided into four classes each of which represented certain false colour (from high to low $F_P$: red, yellow, green, and blue) found within the image of lichen thallus.

Location and area of each of the four Chl fluorescence classes was determined using image analysis system *LUCIA*, as well as the pertinent $F_P$ values from induction curves of Chl fluorescence (*FluorCam* software).

Using the above technique, sub-areas of high to low Chl fluorescence signal were distinguished on the thalli. In the zones exhibiting extremely high and low $F_P$, slow Chl fluorescence induction kinetics were measured, supplemented with a saturation pulse (0.8 s, 5000 µmol m$^{-2}$ s$^{-1}$) at steady-state Chl fluorescence reached after 5 min of irradiance (*PAM-2000, H. W. Walz, Effeltrich, Germany*). The effective quantum yield of photochemical energy conversion in PS2 ($\Phi_2$, $\Phi_{\text{II}}$ for nomenclature see Roháček and Barták 1999) was calculated as $\Delta F/F_M$ (Genty et al. 1989), where $\Delta F$ is the difference between maximum Chl fluorescence after saturation pulse applied on light-adapted lichen sample and steady-state Chl fluorescence. The rate of gross photosynthesis ($P_0$) was then estimated using the formula (Sundberg et al. 1997):

$$P_0 = \text{ETR} \times \Phi_{\text{CO}_2}$$  

(1)

where ETR is electron transport rate and $\Phi_{\text{CO}_2}$ is quantum yield of CO$_2$ fixation. Numeric value of $\Phi_{\text{CO}_2}$ was assumed as 0.125 (Krall and Edwards 1992) considering that minimum 8 quanta are required per one molecule of CO$_2$ fixed. ETR was calculated as:

$$\text{ETR} = \Phi_2 \times 0.5 \times \alpha \times \text{PPFD}$$  

(2)

where 0.5 is a numeric coefficient reflecting the fact that two quanta are required per one electron transported, $\alpha$ is an absorbance of thallii estimated to 0.8, and PPFD is amount of photosynthetically active radiation (actinic irradiance from fluorometer) incident on the top of the thalli.
High irradiance treatment: Fully hydrated thalli of *L. pustulata* and *U. hirsuta* were exposed for 40 min to a PPFD of 2000 μmol m⁻² s⁻¹ in order to induce photoinhibition. Before and after the high irradiance treatment, Chl fluorescence distribution over thalli was measured on dark-adapted (10 min) samples using the method described above. The differences in Chl fluorescence distribution were evaluated using a LUCIA image analysis software. At the thalli area exhibiting maximum *Fₚ*, Chl fluorescence kinetics were recorded before, immediately after, and in 30 min intervals after high irradiance treatment. The kinetics was analysed in order to determine *Fᵥ* changes during high irradiance treatment and subsequent recovery.

**Results and discussion**

**Heterogeneity in Chl fluorescence under full hydration:** There were substantial differences in *Fₚ* over the area of fully hydrated lichen thalli with striking species-specific pattern. In *H. physodes*, the highest *Fₚ* was found in marginal thalli parts and to a lesser extent in few minor parts near the thalli centre (Fig. 1). The finding well fits to the anatomy of *H. physodes*. It is a typical laciniate foliose lichen forming lobes, at the end of which youngest and newly grown thalli parts are located. They can be found either at peripheral thalli parts or within the thalli where they form overlapping structures (Büdel and Scheidegger 1996). In *L. pustulata*, *Fₚ* reached maximum near the central point (umbilicus) and in well-separated spots arranged in irregular circles around it. The distribution of maximum *Fₚ* corresponded well with the anatomic features of the species because the location of the spots showing *Fₚ* maximum was identical with verrucose outgrowths formed at the upper side of the thalli by intercalary pseudomeristems (Honegger 1996). Higher physiological activity at these spots is very probable but some effect of thallus morphology on Chl fluorescence signal could interfere. The edges of verrucose outgrowths were not perpendicularly oriented to the excitation radiation source that could diminish their absorption characteristics and, consequently, Chl fluorescence intensity. In contrast to the other two species, the flat *U. hirsuta* had a patchy distribution of *Fₚ* within the thalli without any regular pattern. Maximum *Fₚ* values were found at relatively large areas, the size and location of which was sample-specific. This may be attributed to a patchy distribution of intercalary pseudomeristems producing new thalli parts that result in irregular intrathalline gradients of physiological activity. This type of heterogeneity is typical for the majority of lichens having umbilicate thallus morphology (Honegger 1993).

The heterogeneity of Chl fluorescence over fully hydrated lichen thall was reflected also in the shape of Chl fluorescence induction kinetics recorded by a modulated fluorometer either at central and marginal parts of thalli. Differences were found in *Fᵥ*, *Fₚ*, and consequently in *Φ₂* values. Since *Φ₂* is the main input parameter for *Pₒ* estimation, there were also differences in *Pₒ* between investigated parts of thalli (Fig. 3).

**Heterogeneity of gross photosynthetic rate (**Pₒ**):** Due to the small size of the thalli of the studied lichen species and the relatively large dimensions of the fluorometric probe, *Pₒ* was studied only at central and marginal parts (Fig. 3). The highest *Pₒ* was found in physiologically more active parts of thalli showing highest *Fₚ*, as indicated previously by an analysis of CFI. In *U. hirsuta*, a difference in *Pₒ* of about 13.3 % was found between physiologically more and less active parts. The difference in *Pₒ* between central and marginal thalli parts of *H. physodes* was less pronounced than in the two other species. This might be attributed to the young age and small size of the thalli that still exhibited a high physiological activity in central parts (values not shown). With pronounced age and size of the *H. physodes* thallus, differences in *Pₒ* between central and marginal thalli parts might be expected. In *L. pustulata*, high *Fᵥ* did not necessarily correspond to a high *Φ₂* and a high *Pₒ*.

**Heterogeneity of Chl fluorescence during desiccation:** In the course of desiccation of thalli from 0 to 90 % WSD all species studied exhibited gradual decrease in *Fₚ* (Fig. 1) throughout whole thalli. This was reflected by a decrease of the thalli area showing the highest Chl fluorescence and by an increase in area of the lowest fluorescence (Fig. 2). At WSD of 90 %, some parts of thallus showed no Chl fluorescence at all and the remaining thalli parts had very low Chl fluorescence. The effect of desiccation was more expressed in a decrease of *Pₒ* value and *Fᵥ* at any time of the Chl fluorescence induction, and in a decrease of maximum Chl fluorescence after a saturation pulse (*Fₘ* derived from Chl fluorescence induction kinetics – data not shown here). These changes are documented for lichens (e.g., Sass et al. 1995, Jensen et al. 1999, Barták et al. 2000a) and explained as consequences of a functional disconnection of PS2 antennae from the PS2 core. *Fₒ* decreased with desiccation which could be caused partially by the changes of optical properties of the upper cortex due to the higher presence of reflective air bubbles (Scheidegger and Schroeter 1995). Other mechanisms contributing to the *Fₒ* decrease with thallus desiccation may be connected with redistribution of excitation radiation to PS1 (Chakir and Jensen 1999).
Fig. 1. Heterogeneity of chlorophyll (Chl) fluorescence over thalli of three lichen species under increasing water saturation deficit [%]. Chl fluorescence is given in false colour in the following order (from maximum to minimum): red, yellow, green, blue.
and with Chl fluorescence re-absorption caused by anten-
nae complex aggregation (Horton et al. 1996) which was
documented for various poikilohydric mosses and lichens
e.g., Bartošková et al. 1999a,b, Takács et al. 2000).

![Graphs showing Chl fluorescence of different lichens](image)

**Fig. 2.** Relative areas taken by false colours (256 colour scale)
within the whole chlorophyll (Chl) fluorescence image (for
source see Fig. 1) of lichen thalli under increasing water
saturation deficit (WSD) during desiccation from a fully
hydrated state. The higher the number of false colours, the
higher the Chl fluorescence signal. WSD for Hypogymnia
physodes: ● 0 %, ○ 70 %, △ 76 %, ▽ 95 %; for Lasallia
pustulata: ● 0 %, ○ 71 %, △ 85 %, ▽ 95 %; for Umbilicaria
hirsuta: ● 0 %, ○ 63 %, △ 72 %, ▽ 96 %.

During desiccation, the species studied showed different
pattern of the loss of Chl fluorescence signal. *H. physodes*
had more or less uniform loss of *F₀* at all thallus parts but
under high dehydration (WSD 95 %) the Chl fluorescence
signal was measurable only in marginal thallus parts. This
pattern probably reflects the distribution of the youngest
thallus parts that are in *H. physodes* located at the end of
lobes. In *L. pustulata*, the fastest decrease of *F₀* during
desiccation was observed at the thallus margin. Under
severe desiccation, only the central part of thallus around
the umbilicus remained active. In *U. hirsuta*, an irregular
pattern of the Chl fluo-rescence signal with ongoing
descission was found. The most sensitive were margins
of the thallus followed by the very central part while the
rest of thalli exhibited some activity even at 80 % of
WSD. This behaviour can be explained by thallus mor-
phology. Marginal thallus parts are thinner than central
parts and thus more susceptible to dehydration. The
central thallus parts were likely capable to retain water for
a longer time and lost their activity later than margins.
Thallus parts showing some physio-logical activity even
at a WSD of 80 % were located in the same zones where
the highest Chl fluorescence signal was detected at full
hydation (cf. Fig. 1).

![Graph showing gross photosynthetic rate (PCO) of different lichens](image)

**Fig. 3.** Gross photosynthetic rate (PCO) at full hydration, CO₂
concentration of 400±5 μmol(CO₂) mol⁻¹, PFFD of 15 μmol m⁻²
s⁻¹, and air temperature of 22 °C for central (C) and marginal
(M) parts in thalli of Hypogymnia physodes and Lasallia
pustulata, and the parts showing high (H) and low (L)
physiological activity in Umbilicaria hirsuta.

Responses to high irradiance: High irradiance treatment
of fully hydrated thalli of *L. pustulata* and *U. hirsuta* led
to a decrease of *F₀* in all thalli parts (see Fig. 4). The
extent of the decrease was, however, species-specific:
when considering the whole thallus, it reached about 75
% of the initial *F₀* value in *L. pustulata* and 97 % in *U.
hirsuta*. *F₀* showed a different response to high irradiance
treatment: it increased in *U. hirsuta* but did not increase
in *L. pustulata*. During recovery from high irradiance
treatment in the dark, *F₀* as well as *Fₐ* did not reach the
initial values even after 60 min (Fig. 4). High irradiance
treatment of *L. pustulata* and *U. hirsuta* led to an
apparent decrease of *Fₐ* which together with the irradiance-
deinduced decrease in *Fₐ* (see Barták et al. 2000b) could be caused by photoinhibitory changes in
photosynthetic apparatus. In *L. pustulata* and *U. hirsuta*,
the *Fₐ* decrease was induced under laboratory conditions
closely simulating outdoor environment at the beginning
of spring (full hydration of thallus, high irradiance). How-
ever, two contradictory opinions exist in the literature
whether the photoinhibitory changes appear in lichens
under field conditions. Kappen et al. (1998) reported that
photoinhibition may not occur under field conditions, especially in high irradiance-adapted lichens. Valladares et al. (1997), on the contrary, reported that photoinhibition was probable in lichen species having a limited pool of xanthophyll cycle pigments. In shade-adapted lichens, undoubtedly, photo-inhibition was documented (Gauslaa and Solhaug 1996, 1999). In our opinion, photoinhibition probably occurs in the field at least under specific combination of some environmental factors (e.g., full thalli hydration, extreme temperature, high irradiance).

In conclusion, we proved the potential of the CFI method as a non-intrusive tool for the analysis of photosynthesis in poikilohydric organisms. In foliose and crustose lichens, the assessment of spatial heterogeneity of Chl fluorescence may help to find a mean thallus activity and location of the area within a lichen thallus that is representative for the whole thallus. When using fluorometry to estimate the apparent electron transport through PS2, effective quantum yield (Φp), or maximum rate of P0, selection of the most suitable and representative area for the placement of the fluorometric probe is crucial. To avoid errors in estimation of physiological status of lichen thallus caused by a non-representative probe placement, CFI should be used prior to spot measurements.

Fig. 4. Fast induction kinetics of chlorophyll fluorescence in Lasallia pustulata (left) and Umbilicaria hirsuta (right) before (A) a high irradiance treatment and 10 (B) or 60 min (C) after it. Kinetics either for the most physiologically active zones (spots) within the thalli (lower panel) and for whole thalli (upper panel).

References

HETEROGENEITY OF CHLOROPHYLL FLUORESCENCE


